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RECENT STUDIES OF CARNIVOROUS PLANTS.[1]

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N. Tischutkin published an article in 1889 in the *Berichte der Deutschen Botanischen Gesellschaft* on the cause of the digestion of albumen by the leaves of *Pinguicula vulgaris* L. in which he endeavors to show that the process of digestion is the result of the action of Bacteria. This is in opposition to the theory of Darwin and other authors that the digestion is analogous to the digestion by means of pepsin in the animal kingdom.

In an article in volume XII of the *Acta Horti Petropolitani* he further discusses the subject and concludes that the pepsin of the leaves of insectivorous plants is not a secretion of the plants themselves but is a by-product formed by the numerous species of Bacteria found in the digestive fluid.

In his former study Tischutkin performed over one hundred and fifty experiments, using extracts from the leaves of Pinguicula and small amounts of the secretions of the insect catching leaves. Into these extracts and secretions he put small cubes of coagulated egg albumen. In no case did there follow any peptonization of the egg albumen. These results impelled him to take up the further study of the relation of micro-organisms to the digestion of albuminous compounds by insectivorous plants, using other species and genera.

[1]Shaw School of Botany, St. Louis. Read before the Botanical Seminar of the University of Nebraska, Feb. 18, 1893.

Certain authors who have investigated the digestive process (among them Darwin, Gorup, Besanz, Reess and Will) have concluded that it is the result of pepsin secreted by the plants. Others, as C. Morren, see only a process of rotting and decay. Tischutkin in his former paper has reviewed the methods by which these various authors have arrived at their results. Morren placed on the leaves of *Drosera binata* Labill. different insects and cubes of coagulated egg albumen and observed that a few hours afterward the albumen became transparent, the angles were rounded off, and after a day or two the pieces entirely disappeared. Microscopic examination of the transparent fragments of albumen revealed the presence of bacteria, monads, and filaments of a mycelium which resembled the conjugating stage of some species of Mucorineæ. He concludes that while this dissolving of the albumen by some absorbent principle is possible, the intervention of pepsin is entirely problematic.

Schimper in the *Botanische Zeitung* for 1882 tells of some experiments which he made with *Sarracenia purpurea.* These are of great value because made on plants growing wild in Massachusetts. A part of the observations were made on insects caught by the plants, and a part by placing pieces of meat on the leaves. He found that on stopping up the mouths of the leaves at their time of opening and thus preventing the free entrance of bacteria the digestion took place no more rapidly than when the bits of meat were placed in pure water, and from this fact he concluded that pepsin was not present. He did not test the liquid by chemical means. He found great numbers of worms in many of the leaves and thinks that they may have something to do in rendering organic matter absorbable.

Hildebrandt in the *Botanische Zeitung* for 1870 tells of the experiments made on the leaves of *Dionæa muscipula* Ell. and on the cups of *Sarracenia, Cephalotus* and *Nepenthes.* He doubts very much whether the insects caught are of value to the plants. "My observations on *Dionæa muscipula* showed me that the long legged spiders which are caught in the leaves exert an injurious influence upon them. A slimy fluid

would be secreted, but after a short time the leaves would commence to die, if I had not removed the spider in time."

Aschman in 1875-6 in his work on insectivorous plants throws doubt on the presence of pepsin in the secretions of Nepenthes and Sarracenia, and believes that the digestive process is simply putrefaction.

Thus we see that many authors have expressed the opinion that the digestion of nitrogenous substances by the secretions of the leaves of insectivorous plants is due to disintegration through the action of the lower organisms rather than by active ferments produced by the plant itself.

Following this preliminary statement of the published data of the subject, Tischutkin takes up his own experiments and gives the results which he has obtained.

The objects which he kept in view while making these experiments were, first, to see whether the presence of lower organisms in the secretions was a constant occurrence; second, if uniformly present, could these organisms dissolve albumen; and, third, to compare the results obtained with other insectivorous plants with *Pinguicula vulgaris*. The plants used were *Pinguicula vulgaris, Drosera longifolia, D. rotundifolia, Dionæa muscipula* and *Nepenthes mastersii*. All of these plants, the last excepted, were cultivated under bell-jars to prevent the visits of insects. To procure the secretion he excited the glands by means of cubes of sterilized egg albumen. The leaves of the first three species responded once to the irritation, those of *Dionæa muscipula*, three times. The acid sap thus obtained was examined microscopically every 24 hours for five days. In every case great multitudes of bacteria were discovered, and frequently there were various moulds as well. The author considers this discovery of much importance, because Darwin had stated that the secretions possess antiseptic properties which prevent the rapid appearance of micro-organisms, and compared their action with that of the gastric juice in the higher animals, which, as is well known, destroys injurious and decay-producing lower organisms.

Gelatine cultures were made in the usual manner, using a drop of the secretion from Dionæa and Drosera 24 hours

after the commencement of the experiment. After the development of from nine to ten generations on Fleisch-Peptone-Gelatine, the different species of bacteria were transferred to test tubes which contained dilute acid peptone-free meat bouillon. Control experiments were made with test tubes containing sterilized water acidulated with dilute hydrochloric acid. Into each test tube was placed a small cube of sterilized egg albumen. In all of those test tubes in which the bacteria developed there followed a rapid solution of the egg albumen commencing at the surface of the cubes and invariably continuing to their centers until solution or peptonization was complete.

By these experiments the author proved that bacteria which possess the power of dissolving egg albumen are always present in the secretions of insectivorous plants.

The number of species that had this power were:

In *Pinguicula vulgaris*, 4 species.
In *Drosera longifolia*, 2 species.
In *Drosera rotundifolia*, 1 species.
In *Dionæa muscipula*, 2 species.
In *Nepenthes mastersii*, 2 species.

His next experiments were made using the leaves of Nepenthes, where comparatively large quantities of the fluid could be procured without special irritation. He examined fully developed pitchers of *Nepenthes mastersii* and *N. kennedyana*. The reaction of the secretion was neutral. The secretion contained neither peptone nor insects, and yet there were vast numbers of micro-organisms. The liquid from a leaf of each species was poured into two test tubes, one acidulated with dilute hydrochloric acid, the other without addition of acid. A small cube of coagulated egg albumen was placed in each test tube, and they were then placed in a thermostat at a temperature of 37.5° C. At the end of sixteen hours the egg albumen in the tubes to which hydrochloric acid had been added, had commenced to dissolve, but in the other two was unchanged. This experiment was made to satisfy himself concerning the truth of observations made by Gorup, Besanz and Will that the secretion of unirritated pitchers possessed the property

of peptonizing egg albumen only after it had passed from neutral to acid reaction. He further says that it is extremely improbable that this peptonizing principle is secreted by the glands of the Nepenthes pitchers, but that it is produced by certain micro-organisms present in the solution. Hence the digestion that takes place is not analogous to digestion of albuminoids in the stomach of man, because in the latter case the secretion of gastric juice follows as a result of direct irritation.

To demonstrate this more fully he repeated his experiments in a modified form, using a neutral secretion which contained no bacteria. For this purpose he selected two unopened cups of *Nepenthes coccinea* and *Nepenthes*——sp. ?——which were so far developed that the liquid was already present in considerable quantity. The leaves were separated from the plant and the petioles were attached to a ring stand so that the pitcher of the leaf was vertical. The outside of each pitcher was disinfected with a freshly prepared 1:1000 sublimate solution in water. After the water of the sublimate solution had partially evaporated, a small hole 3 mm. in diameter was cut in the wall of the pitcher with a pair of sterilized scissors. The secretion from each was then transferred by means of a sterilized pipette into two test tubes each containing two cubic centimeters of distilled water and a small cube of coagulated egg albumen. One of the test tubes was slightly acidulated with hydrochloric acid, the other remained neutral. The four test tubes were then placed in a thermostat at 37.5° C.

At the end of 48 hours there was no solution of the egg albumen, though a control experiment with a test tube containing pepsin gave positive results. Microscopic examination and gelatine cultures gave negative results. These experiments were repeated, using the remainder of the secretion diluted with a little pure glycerin, but the result was the same.

In these experiments the only question that could be raised was whether the pitchers from which the secretion was taken were not too young to contain pepsin. But if one remembers the experiments of Wunschmann in 1872, and also considers

that the cups were more than half filled, there can be little doubt on this ground.

To obviate this objection the experiment was repeated in another way. Two cups were taken, the one from *Nepenthes distillatoria* and the other from *N. hirsuta*, which were still closed but appeared to be just ready to open. Considerable secretion was present. The cups were not separated from the parent plants. The wall of the cup was sterilized with a sublimate solution which was washed away with sterilized water. Then a small V shaped opening was cut in the wall above the surface of the liquid. Through this opening in the wall of the cup a piece of sterilized egg albumen one centimeter long and one millimeter thick was introduced into the cup of *N. hirsuta* and a piece $\frac{1}{4}$ cm. long and 1 mm. thick, into that of *N. distillatoria.* The openings in the walls were then covered with pieces of court plaster, and these for protection from the moisture of the culture house were covered with varnish. After four days the cups opened. The pieces of egg albumen were unchanged, not even the corners being rounded off. Pepsin was not present in the secretion and bacteria only in very small numbers.

The secretion from each cup was then poured out into sterilized test tubes containing small pieces of white of egg, and they were set aside at a temperature of 20-22° C. The white of egg in the secretion from *N. distillatoria* was dissolved in four days, that in the secretion of *N. hirsuta* in five. At the same time enormous quantities of bacteria were developed.

After finishing these experiments the author noticed an article in the *Comptes Rendus* for 1890, by Dubois, who had experimented with the secretions of various Nepenthes species taken before the cups had opened. Dubois's results agree with those of Tischutkin. The secretion taken from the cups just as they were ready to open did not contain pepsin and did not affect the cubes of albumen. But after having been exposed to the air for some time, putrefaction commenced and the resultant liquid contained traces of pepsin. Dubois draws the following conclusions from his experiments.

1st. That the secretion of Nepenthes does not contain any pepsin and that Nepenthes is not an insectivorous plant.

2d. That the phenomenon of disintegration, called "digestion" by Hooker, was without doubt due to the presence of micro-organisms and not to the secretions of the plant itself.

The author finds that the results of his various experiments show that all the disintegration of nitrogenous substances by the leaves of insectivorous plants is *directly dependent* upon the presence of certain lower organisms, principally bacteria, in the secretions of the plants.

The secretion contains bacteria of many different species, micrococci and rod-shapes, and sometimes the mycelia of moulds or other fungi. It is evident that most of these organisms are carried into the cups by the air, though some may be washed in by rain drops or carried on the bodies of insects. The solution of albuminous compounds commences simultaneously with the development of great numbers of micro-organisms in the secretion.

The secretion of the leaves of insectivorous plants is not analogous to the gastric juice of the higher animals. It is only a medium in which bacteria may live and develop while they are breaking up nitrogenous organic insoluble compounds and preparing them for assimilation by the plant.

It will not be uninteresting to present a table showing the chemical composition of the secretion of various Nepenthes species. The calcium and magnesium are in composition with malic and citric acids.

Malic acid and a trace of citric acid	38.61 %
Potassium chloride (Chlorkalium)	50.42
Sodium carbonate	6.36
Calcium	2.59
Magnesium	2.59
Organic matter—a trace	
	100.57.

There are no free acids in the liquid, though Dr. Turner in 1828 reported a trace of oxalic acid.

Without doubt these genera constitute a group entirely unique. They furnish great natural bacteriological laboratories in which the cultivation of microbes which are beneficial to the plant is carried on. It is in a certain sense a symbiosis between the lowest and the most highly organized plants. The higher organisms furnish a food supply for the use of the microbes in order that they may in turn be benefited by food stuffs which the lower organisms manufacture and furnish in convenient form. That the nitrogenous compounds set free by the dissolution of the egg albumen are really absorbed is shown by Darwin in his experiments.

On the ground of his experiments the author offers his conclusions as follows:

1st. The disintegration of albuminous compounds by the secretions of carnivorous plants is due to the growth of micro-organisms, principally bacteria.

2. Micro-organisms possessing the power of dissolving albuminous compounds always vegetate in the secretions of completely developed carnivorous plants.

3. The disintegration of the albumen does not commence at the moment of the secretion of the fluid, but only after micro-organisms have developed in sufficient numbers in the secretion.

4. The micro-organisms found on the leaves of carnivorous plants come principally from the air, though they may be derived from other sources.

5. The name "carnivorous" plants is to be understood in the sense that the plants only assimilate the products which the lower organisms have set free.

6. The rôle of the plant itself is only to furnish a medium in which certain micro-organisms may live and develop.